REGIONAL ASSESSMENT OF OZONE SENSITIVE TREE SPECIES USING BIOINDICATOR PLANTS*

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Abstract. Tropospheric ozone occurs at phytotoxic levels in the northeastern and mid-Atlantic regions of the United States. Quantifying possible regional-scale impacts of ambient ozone on forest tree species is difficult and is confounded by other factors, such as moisture and light, which influence the uptake of ozone by plants. Biomonitoring provides an approach to document direct foliar injury irrespective of direct measure of ozone uptake. We used bioindicator and field plot data from the USDA Forest Service to identify tree species likely to exhibit regional-scale ozone impacts. Approximately 24% of sampled sweetgum (*Liquidambar styraciflua*), 15% of sampled loblolly pine (*Pinus taeda*), and 12% of sampled black cherry (*Prunus serotina*) trees were in the highest risk category. Sweetgum and loblolly pine trees were at risk on the coastal plain of Maryland, Virginia and Delaware. Black cherry trees were at risk on the Allegheny Plateau (Pennsylvania), in the Allegheny Mountains (Pennsylvania, West Virginia, and Maryland) as well as coastal plain areas of Maryland and Virginia. Our findings indicate a need for more in-depth study of actual impacts on growth and reproduction of these three species.

Keywords: air pollution, monitoring, northeastern United States, risk assessment, spatial analysis

1. Introduction

Air pollutants, including ground-level ozone, interact with forest ecosystems (Smith, 1981; Hakkarienen, 1987; Miller and Millecan, 1971). Ozone is the only regional, gaseous air pollutant frequently measured at known phytotoxic levels (Cleveland and Graedel, 1979; Lefohn and Pinkerton, 1988). It causes direct foliar injury to many tree species and has caused reductions in growth and biomass of forest trees in controlled exposure facilities. In the eastern United States, moderately high ozone concentrations and periodic severe exposures occur regularly during the growing season (Skelly, 2000). Ozone exposure is not only an issue in urban areas but also across forested landscapes because of long-range transport of contaminated air masses. Forested landscapes under moderate air pollution dosage

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may have a species-specific response and high dosages may influence ecosystem stability (Smith, 1974).

Plant response to ozone in forested landscapes can be assessed using bioindicator plants (biomonitoring) (Krupa and Manning, 1988). Indicator plants are sensitive species that respond to ambient levels of pollution with typical foliar injury symptoms (Chappelka and Samuelson, 1998; USDA Forest Service, 1999). Monitoring ozone air quality with bioindicator plants does not identify specific levels of ozone present in ambient air but rather identifies whether conditions are favorable for ozone injury to occur. In this sense, bioindicator plants integrate existing environmental conditions (e.g., light, temperature, relative humidity, soil moisture, etc.) that determine actual ozone flux (McCool, 1998).

The USDA Forest Service collects information about ozone air quality on a network of biomonitoring plots (biosites) using ozone sensitive bioindicator plants (trees, woody shrubs, and non-woody herb species). Field protocols are documented in USDA Forest Service (1999). The goal of the ozone biomonitoring network is to provide information about ozone injury to plants in forested land-scapes on regional and national scales. This large-scale monitoring serves as the first step in identifying possible regional or local scale forest ecosystem health issues that may necessitate detailed follow-up investigations.

The objective of this study was to identify forest tree species that are likely to exhibit regional-scale ozone impacts in the northeastern and mid-Atlantic regions of the United States. To accomplish this, the spatial distribution of probable ozone injury to plants was quantified using bioindicator data for the 1994 through 1999 time-period and related to the spatial distribution of forest tree species in the study area.

2. Materials and Methods

We employed the following steps to identify forest tree species likely to exhibit regional-scale ozone impacts. First, information at each biosite was quantified by calculating a biosite index. The biosite index at each biosite was then averaged across years (1994–1999). Next, we used geostatistical procedures to predict the average (1994–1999) biosite index at each USDA Forest Service Forest Health Monitoring (FHM) field plot. Prediction was required because biosites and FHM field plots were not co-located. We then assigned each tree on each FHM field plot the predicted biosite index for their corresponding FHM field plot. Trees were then stratified by species and we calculated average biosite index and created biosite index frequency distributions at the species level. All tree species were then classified as insensitive, moderately sensitive, sensitive, or unknown sensitivity to ozone based on available literature. The average biosite index and frequency distributions for species classified as sensitive were then further examined to identify the four species most at risk. Methods are described in more detail below.



Figure 1. The distribution of Forest Health Monitoring field plots (a) and biosites (b) in the study area.

The study area encompassed Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Virginia, Vermont, Washington, D.C., and West Virginia. There were 599 forested FHM field plots (Figure 1a) with 18841 trees of 78 tree species sampled in 1994–1999. Biosites were located close to or at some distance from the FHM field plots depending on the availability of open areas with ozone bioindicator plants. Areas with little or no canopy were best suited for assessing ozone stress because only plants in openings experience ozone exposures similar to canopy trees (Fredericksen et al., 1995). Bioindicator species including but not limited to blackberry (Rubus allegheniensis Porter), black cherry (Prunus serotina Ehrh.), common milkweed (Asclepias syriaca L.), yellow poplar (Liriodendron tulipifera L.), and white ash (Fraxinus americana L.) were sampled on 512 biosites in the study area (Figure 1b).

At each biosite, between 10 and 30 individual plants of up to three bioindicator species were evaluated for ozone injury. Each plant was rated for the proportion of leaves with ozone injury and the mean severity of symptoms on injured foliage using a modified Horsfall-Barratt scale with breakpoints at 0.06, 0.25, 0.50, 0.75, and 1.0 (Horsfall and Cowling, 1978; USDA Forest Service, 1999). We used these data to calculate a biosite index (*BI*) (Smith, 1995) for each plot, for each measurement year.

$$BI = 1000 \left(m^{-1} \sum_{j=1}^{m} n_j^{-1} \sum_{i=1}^{n_j \ge 10} a_{ij} s_{ij} \right)$$

where

BI = biosite index;

m =number of species evaluated;

 n_i = number of plants of the jth species evaluated;

 a_{ij} = proportion of injured leaves on the *i*th plant of the *j*th species;

 s_{ij} = average severity of injury on the *i*th plant of the *j*th species.

The biosite index was the average score (amount * severity) for each species averaged across all species on the biosite multiplied by 1000 to allow risk categories to be defined by integers. We classified the biosite index values into four risk categories (Table I) based on groupings proposed by Smith (1995). The groupings were based on expert interpretation of preliminary field studies (1990–1994) and were designed to capture differences in plant damage to ozone sensitive species in areas of low, moderate, and high ozone exposure (Lewis and Conkling, 1994). The 'risk' assigned to each category represents a relative measure of impacts from ambient ozone exposure (Table I).

The number of measurement years per biosite varied from 1 to 6. Some biosites in Massachusetts and Maine had six measurements while New York biosites were

TABLE I
Biosite index categories, risk assumption, and possible impact

Biosite Index Category		Assumption of risk	Possible impact		
1.	Biosite index = $0 \le 5$	None	Tree-level response		
	Little or no foliar injury		Visible injury to leaves and needles		
2.	Biosite index = $5.0 \le 15$	Low	Tree-level response		
	Low foliar injury		Visible and invisible injury		
3.	Biosite index = $15 \le 25$	Moderate	Tree-level response		
	Moderate foliar injury		Visible and invisible injury		
4.	Biosite index > 25	High	Structural and functional changes		
	Severe foliar injury		Visible and invisible injury		

measured in 1999 only. The average biosite index for all measurements (1994–1999) was used as the biosite index in subsequent analyses.

Kriging was used to assign a biosite index to each FHM field plot. Spatial autocorrelation between biosites was examined for anisotropy and structure using directional variograms (Isaaks and Srivastava, 1989) and theoretical variograms were constructed for both the North-South and East-West directions using a Gaussian and exponential model, respectively. Ordinary kriging estimates of the biosite index were calculated based on a nested model to account for the different spatial relationships in the North-South and East-West directions and were made for each FHM field plot in the study area. For illustrative purposes, we interpolated a surface of mean biosite index values for the study area using block kriging procedures (Isaaks and Srivastava, 1989). We interpreted kriging estimates in a probabilistic sense. For example, areas with a high estimated biosite index value were more likely to be experiencing favorable conditions for injury to plants from ozone.

Each tree greater than 2.54 cm in dbh (diameter at breast height – 1.37 m) on each FHM field plot was assigned the biosite index estimate for the plot. We calculated the average biosite index and created frequency distributions for each tree species in the multi-state study area with at least 20 individuals. Each tree species was stratified by its sensitivity (sensitive, moderately sensitive, or insensitive) based on the most recently published sensitivity lists (Krupa and Manning, 1988; Krupa et al., 1998; Skelly, 2000; Skelly et al., 1987; Smith, 1981) or field reports (Eckert et al., 1994; Hildebrand et al., 1996; Renfro, 1992) using ambient exposure levels (Table II). Sensitive tree species were the focus of this analysis. Ozone sensitive tree species with the four highest mean biosite index values and 20 or more individuals present were selected for further analysis. We then identified where each of the species were at risk.

TABLE II

Mean biosite index, ozone sensitivity, the number of sample trees, and the number of plots for each tree species in the study area

Tree species		Sensitivity	Citation	Mean biosite index		Number of trees
Balsam fir	Abies balsamea	InSen ^b	Smith, 1981	1.2	121	1231
Boxelder	Acer negundo	ModSen ^b	Smith, 1981	10.7	6	17
Striped maple	Acer pensylvanicum	Unk		5.8	63	168
Red maple	Acer rubrum	Sen	Eckert et al., 1994	6.6	440	3183
Silver maple	Acer saccharinum	Unk		0.0	2	22
Sugar maple	Acer saccharum	InSen	Renfro, 1992	6.3	209	1300
Mountain maple	Acer spicatum	Unk		1.7	16	32
Ohio buckeye	Aesculus glabra	Unk		3.0	2	3
Serviceberry	Amelanchier arborea	Sen	Renfro, 1992	23.8	25	46
Pawpaw	Asimina triloba	Unk		12.3	6	18
Yellow birch	Betula alleghaniensis	Sen	Renfro, 1992	4.9	153	560
Sweet birch	Betula lenta	Unk		13.2	88	340
Paper birch	Betula papyifera	ModSen	Eckert et al., 1994	1.9	109	489
GTray birch	Betula populifolia	ModSen	Eckert et al., 1994	6.3	24	117
Bitternut hickory	Carya cordiformis	Unk		7.8	22	34
Pignut hickory	Carya glabra	Unk		7.6	62	163
Shagbark hickory	Carya ovata	Unk		6.5	33	97
Hickory sp.	Carya sp.	Unk		7.1	22	52
Mockernut hickory	Carya tomentosa	Unk		10.1	41	104
Hackberry	Celtis occidentalis	Unk		19.5	3	3
Eastern redbud	Cercis canadensis	ModSen	Renfro, 1992	7.5	9	17
Flowering dogwood	Cornus florida	ModSen	Renfro, 1992	8.4	53	87
Hawthorn	Crataegus sp.	Sena	Krupa et al., 1998	21.7	6	14
Common persimmon	Diospyros virginiana	Unk		9.0	4	5
American beech	Fagus grandifolia	Unk		4.7	180	896
White ash	Fraxinus americana	Sen	Skelly, 2000	7.2	146	511
Black ash	Fraxinus nigra	Sen ^a	Krupa et al., 1998	1.4	14	45
Green ash	Fraxinus pennsylvanica	Sen	Krupa and	3.5	20	49
			Manning, 1988			
American holly	Ilex opaca	InSen ^b	Smith, 1981	25.1	14	61
Black walnut	Juglans nigra	Unk		6.7	14	31
Eastern redcedar	Juniperus virginiana	Unk		8.9	23	46
Tamarack (native)	Larix laricina	Unk		0.1	6	18
Sweetgum	Liquidambar stryraciflua	Sen	Krupa et al., 1998	17.7	40	202
Yellow-poplar	Liriodendron tulipifera	Sen	Krupa and	10.1	105	469
• •			Manning, 1988			
Cucumbertree	Magnolia acuminata	Unk		9.8	12	29
Apple sp.	Malus sp.	Unk		4.1	18	51
Blackgum	Nyssa sylvatica	ModSen	Renfro, 1992	15.2	87	231
Sourwood	Oxydendrum arboreum	ModSen	Renfro, 1992	8.3	26	74
Norway spruce	Picea abies	InSen ^b	Smith, 1981	0.1	8	119

Based on relative sensitivity of genus not species.
 Based on relative sensitivity to acute ozone exposure.

TABLE II (continued)

Tree species		Sensitivity	Citation	Mean biosite index	Number of plots	Number of trees
	D' I	InSen ^b	Consists 1001	0.8	30	78
White spruce	Picea glauca	Unk	Smith, 1981	0.8	30 11	109
Black spruce	Picea mariana	InSen	E-1 1004	1.8	107	1004
Red spruce	Picea rubens	ModSen ^b	Eckert et al., 1994	9.9	107	31
Shortleaf pine	Pinus echinata		Smith, 1981		4	31 19
Table mountain pine	Pinus pungens	Sen v.a. b	Renfro, 1992	29.8		
Red pine	Pinus resinosa	InSen ^b	Smith, 1981	2.8	10	26
Pitch pine	Pinus rigida	InSen	Eckert et al., 1994	5.8	16	152
Eastern white pine	Pinus strobus	Sen	Krupa and	2.9	127	969
			Manning, 1988		_	
Scotch pine	Pinus sylvestris	ModSen ^b	Smith, 1981	10.8	5	60
Loblolly pine	Pinus taeda	Sen	Taylor, 1994	20.4	27	431
Virginia pine	Pinus virginiana	ModSen	Renfro, 1992	11.7	33	259
Sycamore	Platanus occidentalis	Sen	Krupa and	8.0	9	14
			Manning, 1988			
Balsam poplar	Populus balsamifera	Sena	Krupa et al., 1998	0.9	4	8
Eastern cottonwood	Populus deltoides	Sen ^a	Krupa et al., 1998	0.8	4	16
Bigtooth aspen	Populus grandidentata	Sen ^a	Krupa et al., 1998	2.2	27	74
Quaking aspen	Populus tremuloides	Sen	Krupa and	1.6	76	306
			Manning, 1988			
Pin cherry	Prunus pensylvanica	ModSen	Renfro, 1992	2.1	17	39
Black cherry	Prunus serotina	Sen	Krupa and	13.2	154	521
			Manning, 1988			
Chokecherry	Prunus virginiana	ModSen	Renfro, 1992	52.1	5	13
White oak	Quercus alba	InSen	Renfro, 1992	9.7	126	431
Scarlet oak	Quercus coccinea	ModSenb	Smith, 1981	13.4	50	136
Northern pin oak	Quercus ellipsoidalis	ModSenb	Smith, 1981	10.2	1	1
Southern red oak	Quercus falcata	Unk		14.8	25	62
Shingle oak	Quercus imbricaria	InSen ^b	Smith, 1981	0.0	1	1
Bur oak	Quercus macrocarpa	Unk		0.0	1	2
Pin oak	Quercus palustris	ModSenb	Smith, 1981	5.2	3	4
Willow oak	Quercus phellos	Unk		13.8	12	27
Chestnut oak	Quercus prinus	Unk		10.6	88	621
Northern red oak	Quercus rubra	InSen	Eckert et al., 1994	10.3	183	639
Post oak	Quercus stellata	Unk		14.2	11	13
Black oak	Quercus velutina	ModSenb	Smith, 1981	10.3	88	228
Black locust	Robina pseudoacacia	ModSen	Renfro, 1992	7.6	35	117
Black willow	Salix nigra	Unk		0.0	3	8
Sassafras	Sassafras albidum	Sen	Krupa et al., 1998	9.1	41	111
Northern white-cedar	Thuja occidentalis	InSen	Eckert et al., 1994	0.4	45	409
American basswood	Tilia americana	InSen ^b	Smith, 1981	4.7	35	75
Eastern hemlock	Tsuga canadensis	InSen	Renfro, 1992	2.9	114	782 .
American elm	Ulmus americana	Unk	,	5.5	27	80
Slippery elm	Ulmus rubra	Unk		8.4	15	31
Subhera emi	Omas raviu	OHK.		0.7	10	

^a Based on relative sensitivity of genus not species.
^b Based on relative sensitivity to acute ozone exposure.

3. Results

Most of the trees on the 599 forested field plots in the study area were not at risk to ozone injury. Approximately 64% of the plots in the study area experienced conditions unfavorable for ozone injury (Table I, category 1). Twenty-two percent of the plots in the study area had low risk (Table I, category 2). Eight percent of the plots had moderate risk (Table I, category 3), and only 6% were at high risk (Table I, category 4). However, we found certain geographic areas to be more at risk than others.

Most of New York and northern New England experienced conditions under which plant injury from ozone would not be expected (Figure 2). Conversely, the Allegheny Mountains (PA, MD, and WV) and the Allegheny Plateau (PA) experienced conditions where plant injury from ozone was expected. The highest estimated biosite index values were found on the Allegheny Plateau region of Pennsylvania and relatively high values were also found in Delaware, near the Chesapeake Bay, and coastal plain areas of Maryland and Virginia (Figure 2).

Nineteen tree species in the study area were classified as ozone sensitive (Table II). Sensitive tree species with a mean biosite index of less than 5 (no risk) were Eastern white pine (Pinus strobus), green ash (Fraxinus pennsylvanica), quaking aspen (Populus tremuloides), and yellow birch (Betula alleghaniensis). Seventy-three to 94% of these species occurred in areas where conditions were unfavorable for plant injury from ozone (Figure 3a). Red maple (Acer rubrum), sassafras (Sassafras albidum), and white ash (Fraxinus americana) had mean biosite indexes of 6.6, 9.1, and 7.2, respectively. Seventy-three percent of white ash, 71% of red maple, and 47% of sassafras trees occurred in areas where conditions were unfavorable for ozone injury (Figure 3b). However, the majority of sassafras trees were in areas with some degree of risk (categories 2-4). Black cherry and yellow poplar had mean biosite index values of 13.2, and 10.1, respectively. Approximately 12% of black cherry and 8% of yellow poplar trees occurred in areas where conditions were favorable (biosite index > = 25) for plant injury from ozone and were at high risk (Figure 3c). Sampled loblolly pine (Pinus taeda), sweetgum (Liquidambar styraciflua), and serviceberry (Amelanchier arborea) trees had mean biosite index values between 15 and 25 (moderate risk). Approximately 15% of the sampled loblolly pine, 24% of sweetgum, and 26% serviceberry trees had biosite index values greater than 25 and were at high risk for ozone injury (Figure 3d). Ozone sensitive tree species with the four highest mean biosite index values and 20 or more individuals were black cherry, loblolly pine, sweetgum, and serviceberry.

FHM field plots with black cherry present and biosite index values greater than 15 (moderate to high risk) occurred along the Allegheny Mountains, on the Allegheny Plateau, and along the coastal areas of Maryland and Virginia (Figure 4a). Loblolly pine trees at moderate to high risk occurred in on the coastal plain of Maryland and Virginia (Figure 4b). This was also the case with sweetgum trees (Figure 4c). FHM field plots with serviceberry present and biosite index values

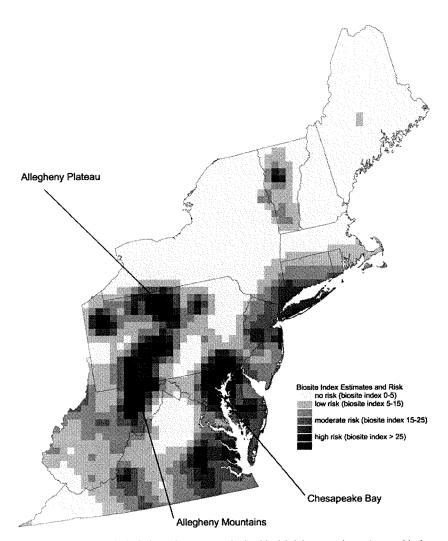


Figure 2. Interpolated biosite index estimates created using block kriging procedures. Average biosite index was calculated for a lattice of 400 sqkm cells based on kriged estimates for sixteen points in each cell.

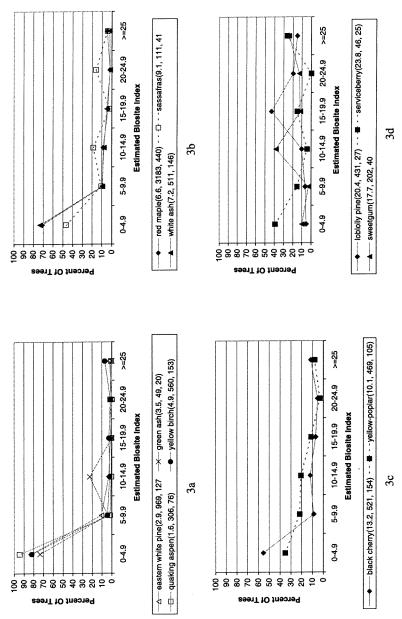


Figure 3. Frequency distributions for ozone sensitive tree species in the study area. The legend identifies the species name, mean biosite index, number of sample trees, and the number of plots.

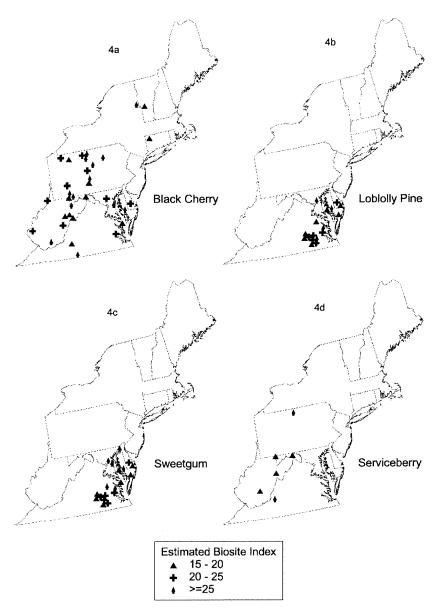


Figure 4. Distribution of FHM field plots with an estimated biosite index greater than 15 and black cherry (a), loblolly pine (b), sweetgum (c), and serviceberry (d) trees.

greater than 15 occurred along the Allegheny Mountains and on the Allegheny Plateau (Figure 4d).

4. Discussion

Ozone can directly impact tree growth, forest succession, forest species composition, and causes visible injury on some forest tree species (Hakkarienen, 1987; Miller and Millecan, 1971; Skelly *et al.*, 1987; Treshow and Stewart, 1973). There may be secondary impacts on forest dependent wildlife, insects and pathogens. Economic impacts are also possible if growth rates of commercially important tree species are reduced. The genetic base of species with a genetically variable response to ozone may also be impacted. Specifically, certain genes or gene complexes could be lost in a relatively short time-period and the population's genetic base could be narrowed if sensitive genotypes occur in areas that experience favorable conditions for plant injury from ozone (Bennett *et al.*, 1994).

Black cherry, loblolly pine, and sweetgum are key species both economically and ecologically in the areas they were predicted to be at risk. Black cherry is a commercially important species on the Allegheny Plateau and its fruit is important to wildlife such as squirrels, deer, turkey, nongame birds, mice and moles throughout the native range (Burns and Honkala, 1990a). It is a component of many northern hardwood stands and is the primary species in the Black Cherry-Maple forest type associated with the Allegheny Plateau and Allegheny Mountains of Pennsylvania, New York, Maryland, and West Virginia. Loblolly pine and sweetgum are both commercially important species where they occur in the southeast part of the study area. Loblolly pine is a major component of pine and pine-hardwood stands. These stand types provide habitat for a variety of game and nongame wildlife species (Burns and Honkala, 1990b). Sweetgum seeds are a food source for several bird species, squirrels, and chipmunks (Burns and Honkala, 1990a).

Serviceberry also frequently occurs in areas predicted to experience conditions conducive to ozone injury to plants. However, this species is generally a minor component in the understory of mountain forests (Brown and Kirkman, 1990). Since serviceberry is an understory species, it may not be experiencing the predicted conditions because the forest canopy may be serving as an effective air filter of phytotoxic ozone concentrations (Treshow and Stewart, 1973; Skelly *et al.*, 1996).

Southern red oak (*Quercus falcata*) and sweet birch (*Betula lenta*) had estimated biosite indexes high enough to warrant concern (Table II). Eighteen percent of sampled southern red oak trees and 16% of sampled sweet birch trees were predicted to be at high risk. However, their sensitivity to ozone was unknown. Sweet birch is of particular concern because other *Betula* sp. in the study area were classified as either sensitive or moderately sensitive to ozone. We could not evaluate

the risk of regional-scale ozone injury to these species without better information on their sensitivity to ozone.

Foliar response to ambient ozone concentrations was used to assign sensitivity rankings for tree species discussed in this report and to extend this discussion into the area of regional-scale ozone impacts in northeastern and mid-Atlantic forests. The use of ozone sensitive terminology can be problematic as there is no consistent relationship between visible injury and growth. A tree species ranked as ozone sensitive based on foliar response may exhibit no measurable adverse effect on growth-related processes. However, a number of studies indicate that ambient ozone exposures high enough to cause visible symptoms can be directly related to growth losses in some species, for example, white pine (Benoit *et al.*, 1982; Chappelka and Samuelson, 1998). Similarly, Chevone (2001) reports a strong inverse relationship between photosynthetic activity and visible leaf injury in field-grown black cherry. Field studies using bioindicator plants to identify biologically critical ozone exposures may help reveal some of the complex relationships between visible and invisible injury on native vegetation and so better characterize the sensitive response.

The results of this study indicated that four tree species are possibly at risk on a regional scale from ambient levels of ozone. All except serviceberry are shade intolerant, upper-canopy species and therefore more likely exposed to ozone deposition. These results suggest that an in-depth study of actual impacts on growth and reproduction is warranted for black cherry, loblolly pine, and sweetgum because cause-effect relationships are difficult to assess with large-scale biomonitoring data (Schreuder and Thomas, 1991). Results also indicated that sweet birch and southern red oak were experiencing conditions on a regional scale where injury from ozone was possible, but a better definition of their sensitivity is needed.

Finally, there is a recognized need for improvement in the national secondary ozone standard to protect the forest resource. Due to the complexity of ozone exposure-response relationships, linking air quality data to a biological interface remains a challenge. Recent assessment studies have examined various exposure indices and simulation models to predict forest response to ozone. The approach presented here tends to confirm the findings of Hogsett *et al.* (1994) and Lefohn *et al.* (1997) that regional ozone concentrations may be having an impact on sensitive tree species in eastern forests. However, the specific results for New York are based on only one year of data and should be evaluated after additional data are available. The use of a region-wide biomonitoring network and a biosite index averaged over several years with variable weather and ozone regimes provides new, biologically relevant information that should improve assessment models and help address ozone policy issues regarding forest health protection.

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